Bioorganic & Medicinal Chemistry Letters 19 (2009) 6922–6925

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

COX, LOX and platelet aggregation inhibitory properties of Lauraceae neolignans

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ARTICLE INFO

Article history:
Received 27 September 2009
Revised 14 October 2009
Accepted 15 October 2009
Available online 20 October 2009

Keywords:
Lauraceae
Neolignans
Bicyclo[3.2.1]octane
Benzofuran
COX
5-LOX
Platelet aggregation

ABSTRACT

The anti-inflammatory potential of 26 neolignans (14 of the bicyclooctane-type and 12 of the benzofuran-type), isolated from three Lauraceae species (*Pleurothyrium cinereum, Ocotea macrophylla* and *Nectandra amazonum*), was evaluated in vitro through inhibition of COX-1, COX-2, 5-LOX and agonist-induced aggregation of rabbit platelets. Benzofuran neolignans were found to be selective COX-2 inhibitors, whereas bicyclooctane neolignans inhibit selectively the PAF-action as well as COX-1 and 5-LOX. The neolignan 9-*nor*-7,8-dehydro-isolicarin B **15** and cinerin C **7** were found to be the most potent COX-2 inhibitor and PAF-antagonist, respectively. Nectamazin C **10** exhibited dual 5-LOX/COX-2 inhibition.

Cyclooxygenase (COX) and lipoxygenase (LOX) are the two major enzyme families that catalyze the rate-limiting step in the formation of prostanoids, prostaglandins (PGs), and thromboxane A_2 (TxA_2) by the COX pathway, and leukotrienes (LTs) by the LOX pathway, whose products are significant mediators of pain, fever and inflammation.[1] Inflammatory effects result both from direct actions of PGs and LTs (on the microvasculature, on nociceptive afferents and on temperature-regulating centers in the hypothalamus) and, indirectly, by synergy with other inflammatory mediators including bradykinin, histamine, activated component and platelet activating factor (PAF).[2] There are three isoforms of COX, namely a constitutive form (COX-1) that is present in many tissues such as platelets, stomach, lungs, kidneys etc, an inducible form (COX-2) that is expressed during inflammation as a result of stimulation by cytokines, nitric oxide and growth factors, and a splice variant of COX-1 (COX-3).[2,3] A great interest in developing inhibitors specific for COX-2 had increased in the last years, expecting that it will lack the adverse effects caused by the inhibition of COX-1 enzyme. However, considering the pro-inflammatory properties of prostanoids, a current interest had been focused for dual 5-LOX and COX-2 inhibitors, which had emerged as a rational advance for the design of efficacious anti-inflammatory agents.[1,4]

It has been demonstrated that platelets play an important role in acute inflammation.[5] They accumulate and respond to injury by releasing important mediators such 5-HT, PGs, PAF and hydrolases.[6] Furthermore, TxA_2, formed by platelets has been reported to be potent constrictor of blood vessels and an aggregator of platelets,[7] which are activated by several chemical agents such as arachidonic acid (AA), adenosine diphosphate (ADP), collagen, epinephrine, platelet activating factor (PAF), among others.[8] PAF was discovered to be a lipid mediator of hypersensitivity and inflammation.[9] Several studies have implicated PAF in such diseases as asthma, hypertension, cardiac anaphylaxis and arthritis as well as its clinical benefits on these cases.[10]

As part of our search for bioactive neolignans from Lauraceae plants, a phytochemical exploration was carried out on the leaves of *Pleurothyrium cinereum*, *Ocotea macrophylla* and *Nectandra amazonum*, afforded 26 neolignans, 14 related to the bicyclooctane-type (cinerins A-D[11] **1**–**4**, ocophyllols A-C[12] **5**–**7**, nectamazins A–C[13] **8**–**10**, kadsurenin C[13] **11**, 4′-oxo-macrophyllin B[14] **12**, macrophyllin B[14] **13** and 2′-*epi*-guianin[12] **14**), and 12 related to the 8.5′,7.O.4′-connected (9′-*nor*-7,8-dehydro-isolicarin B[15] **15**, (−)- and (+)-licarin B[12,14] **16**–**17**, (−)- and (+)-licarin A[13,14] **18**–**19**, (+)-mirandin A[14] **20**, ocophyllals A-B[12] **21**–**22**, (+)-acuminatin[13] **23**, (+)-denudatin B[13] **24**, (+)-kadsurenone **25** and liliflol A **26**). The structures and the absolute configuration (Figs. 1 and 2) of the former compounds were determined by extensive spectroscopic analyses, whose isolation and structural elucidation is discussed in previous papers.[11–15]

Several Lauraceae plants have exhibited anti-inflammatory properties.[16–19] The Lauraceae chemistry is recognized to comprise

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doi:10.1016/j.bmcl.2009.10.069

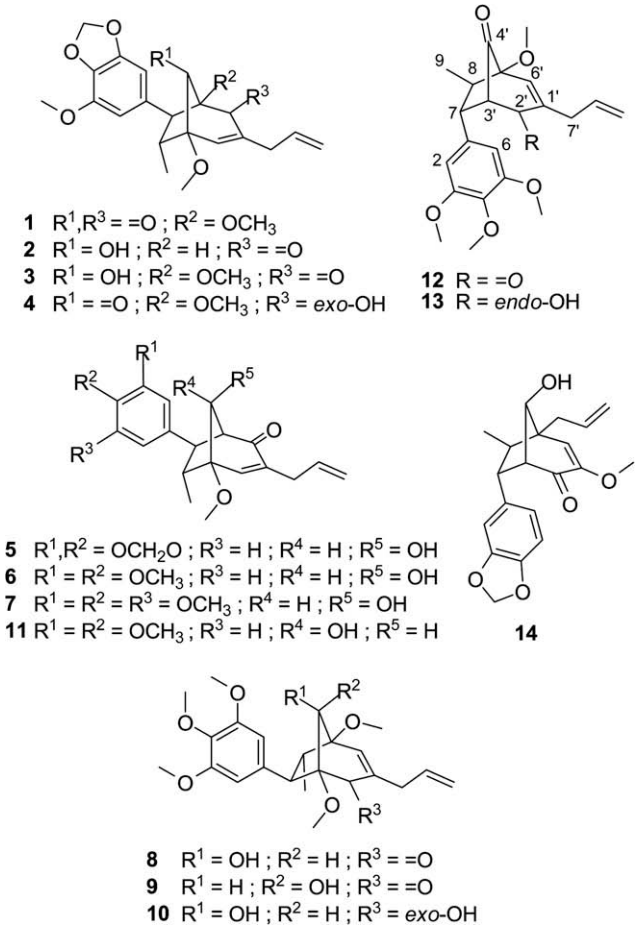

Figure 1. Structures of the bicyclo[3.2.1]octane neolignans.

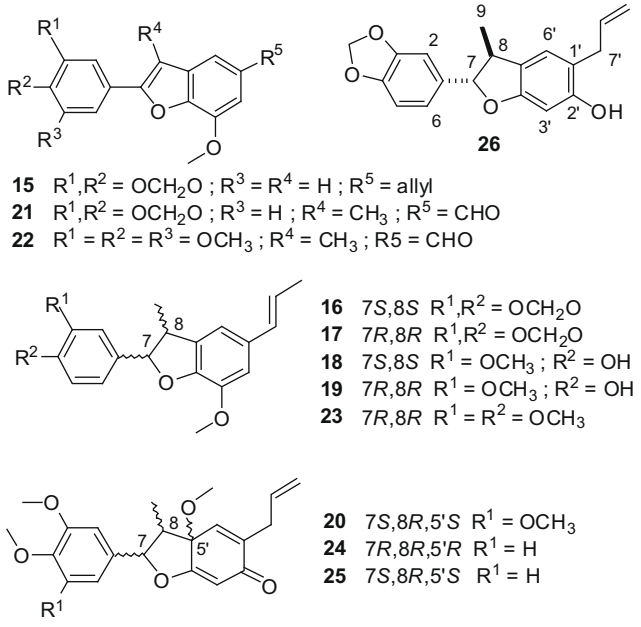

Figure 2. Structures of the 8.5′,7.O.4′-connected neolignans.

neolignans, mostly related to the 8.5′,7.O.4′-connected class, which have shown in vitro anti-inflammatory activity through suppression of tumor necrosis factor (TNF)-α and nitric oxide (NO) production,[20] and inhibitory activity against the two isozymes of COX.[21]

On the basis of these facts and in order to establish the potential as anti-inflammatory agents of the isolated compounds, an in vitro screening for the capability to inhibit the cyclooxygenase (COX) isozymes (COX-1 and COX-2) and 5-lipoxygenase (5-LOX) were accomplished, using a COX-(ovine) and a 5-LOX-(potato) inhibitor screening kits, respectively, following the reported methodologies.[22]

The in vitro abilities (IC_{50} values, μM) of the isolated compounds (**1**–**26**) to inhibit the isozymes COX-1 and COX-2 were determined in the COX-catalyzed transformation of AA into PGH_2, which is then reduced to $PGF_{2\alpha}$ and detected by an enzyme immunoassay (EIA). Although only a work reported the COX-1 and COX-2 inhibition of four bicyclooctane diastereomers (isolated from the stem bark of *Ocotea bullata*, a medicinal plant from southern Africa), which exhibited no inhibitory effect,[23] the neolignans **1**–**14** showed COX-inhibition at different levels (Tables 1 and 2). The macrophyllin-type[14] neolignans **2**–**3**, **5**–**7**, **11**, **14** as well as the guianin-type[14] neolignan **14** exhibited selectivity toward COX-1 inhibition (IC_{50} values 18.2–94.9 μM range).

Compounds **2** and **11** (which have identical bicyclooctane moiety, being solely differenced by the aryl-substitution) have similar IC_{50} values (32.5 and 38.7 μM, respectively), suggesting that the aryl-substitution was not a significant structural condition in the COX-1 inhibition for this type of neolignans, although 3,4,5-trioxyphenyl neolignans showed slightly higher inhibition than 3,4-dioxyphenyl neolignans, as shown for compounds **5**–**7**. However, the activity was notably influenced by the configuration of the bicyclooctane moiety. This statement is supported on comparing the activity for the C-4′-epimers **8** and **9**, which possess a C-4′ hydroxyl group placed in opposite orientation. Compound **8** (with OH group oriented toward aryl group) exhibited a higher IC_{50} value to that of **9** (with OH group oriented toward enone). Similar result was observed between compounds **2** and **6**.

Although the neolignans with a carbonyl group at C-4′ (like **1**, **4**, **12**–**13**) instead C-4′ OH group showed weaker COX-1 inhibitory activity, compounds having a C-2′ hydroxyl group (like **4**, **10** and **13**) showed selective COX-2 inhibition, which is supported on comparing the IC_{50} values of the compounds **12** and **13**, whose only difference is the functional group at C-2′ (C-2′ carbonyl group in **12** and C-2′ *endo*-hydroxyl group in **13**). Compounds **10** and **14** were found to be the most potent COX-1 and COX-2 inhibitors (IC_{50} 18.2 and 6.83 μM, respectively), among the bicyclo[3.2.1]octane neolignans.

Table 1
COX-1, COX-2 and 5-LOX enzyme inhibition of bicyclo[3.2.1]octane neolignans **1**–**14**

Compds	IC_{50}[a] (μM)		SI[b]	IC_{50}[a] (μM)
	COX-1	COX-2		5-LOX
1	165	288	0.573	45.6
2	32.5	215	0.151	146
3	28.3	356	0.0795	136
4	188	92.1	2.04	8.84
5	88.6	312	0.284	256
6	94.9	288	0.330	189
7	72.1	245	0.294	176
8	72.2	255	0.283	156
9	152	569	0.266	42.4
10	74.6	6.83	10.9	12.8
11	38.7	312	0.124	117
12	356	844	0.422	92.5
13	216	65.4	3.30	18.9
14	18.2	326	0.0558	113
Celecoxib	8.32	0.113	73.4	11.3
Aspirin	0.411	2.53	0.162	—
Caffeic acid	—	—	—	3.74

[a] Values are means of two experiments, standard deviation from the mean is <10% of the mean value.
[b] In vitro COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2).

Table 2
COX-1, COX-2 and 5-LOX enzyme inhibition of 8.5′,7.O.4′-connected neolignans **15**–**26**

Compds	IC_{50}[a] (μM)		SI[b]	IC_{50}[a] (μM)
	COX-1	COX-2		5-LOX
15	349	3.32	106	329
16	366	25.6	14.3	426
17	289	52.1	5.55	157
18	312	32.1	9.73	35.5
19	245	66.4	3.69	12.5
20	77.9	126	0.620	13.2
21	123	16.8	7.31	436
22	102	12.7	8.02	412
23	223	28.7	7.75	146
24	88.6	446	0.199	15.6
25	25.6	246	0.104	12.6
26	179	98.7	1.81	10.2
Celecoxib	8.32	0.113	73.4	11.3
Aspirin	0.411	2.53	0.162	—
Caffeic acid	—	—	—	3.74

[a] Values are means of two experiments, standard deviation from the mean is <10% of the mean value.
[b] In vitro COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2).

In the case of the COX inhibitory activity displayed by the 8.5′,7.O.4′-connected neolignans, it was found that the neolignans having a dihydrobenzofuran core (**16**–**19**, **23**, **26**) selectively inhibited the COX-2 enzyme at moderate level (IC_{50} 25.6–98.7 μM range). The levorotatory dihydrobenzofuran neolignans **16** and **18** were found to be slightly more active than the dextrorotatory compounds **17** and **19**.

The neolignans having a benzofuran moiety (**15**, **21**–**22**) also exhibited COX-2 selectivity, affording the best results (IC_{50} 3.32–16.8 μM range). Thus, compound **15** was the most active COX-2 inhibitor (IC_{50} 3.32 μM), among neolignans **1**–**26**. Although **15** was much weaker COX-2 inhibitor than the selective COX-2 inhibitor celecoxib (1.5% of the celecoxib's potency), the COX-2 selectivity index of **15** was found to be higher (COX-2 SI = 106), suggesting further studies toward structural optimization for increasing the activity, perhaps by preparation of derivatives of **15** containing the COX-2 pharmacophores methanesulfonyl ($MeSO_2$) or sulfonamide (H_2NSO_2) as substitutions.[4,22] In contrast, the neolignans with dihydrobenzofuran-(2*H*)-one moiety (**20**, **24**–**25**) inhibited selectively the COX-1 action.

In vitro 5-LOX enzyme inhibition studies indicate that compounds **4**, **10**, **13**, **19**–**20**, **24**–**26** had comparable 5-LOX inhibition activity than celecoxib, but lesser than caffeic acid. Interestingly, compound **10** had a dual 5-LOX/COX-2 inhibition (COX-2 IC_{50} 6.83 μM; 5-LOX IC_{50} 12.8 μM), which actually is the main intention for the development of new anti-inflammatory agents. This dual inhibition of **10** might be likely due to the ability for binding to, or chelate iron present in the 5-LOX enzyme, by their groups at C-2′ and C-3′, whose orientation places them spatially nearby. Thus, **10** is a good candidate to be used as dual inhibitor in further anti-inflammatory studies. As expected, neolignans having a free phenolic OH group (**18**, **19**, **26**) showed 5-LOX inhibition, but their COX-inhibition was no significant. The dihydrobenzofuran-(2*H*)-one neolignans (**20**, **24**–**25**) were found to be equipotent (IC_{50} values in the 12.6–15.6 μM range). However, further structure–activity studies and biological analyses are required to clarify the underlying mechanism and to draw unambiguous conclusions for the COX-inhibition for these classes of neolignans.

The capability to inhibit the agonist-induced platelet aggregation had been previously evaluated for lignans and neolignans induced by agonists such as platelet activating factor (PAF), arachidonic acid (AA), adenosine 5′-diphosphate (ADP), adrenaline, among others.[24–26] In addition, some 8.5′,7.O.4′-connected and bicyclo[3.2.1]octane neolignans isolated from Chinese medicinal plants were found to be potent PAF-antagonists in the ^{3}H-PAF receptor binding assay.[27] In previous papers, we report the inhibition of PAF-induced platelet aggregation of neolignans **1**–**11**.[11–13] However, in order to establish selectivity toward the common agonist identified in platelet aggregation, it is also described herein a comparison of the abilities to inhibit the platelet aggregation induced by PAF (7.20 nM), AA (100 μM), and ADP (4.00 μM) for the neolignans **1**–**26** (Table 3), following the reported methodology.[10]

Clear trends were observed in this screening. ADP-induced platelet aggregation was no significantly inhibited by the evaluated compounds **1**–**26**. Macrophyllin-type bicyclooctane neolignans **1**–**13** showed a noticeable selectivity toward PAF-inhibition on comparing the IC_{50} values when the other two agonists were used, whilst compound **14** (a guianin-type neolignan) was found to be a non-selective inhibitor. Macrophyllin-type neolignans having a C-4′ hydroxyl group (like **2**–**3**, **5**–**11**, **14**) exhibited higher inhibition toward PAF (IC_{50} in the 1.09–3.78 μM range values) than neolignans with C-4′ keto group (IC_{50} in the 6.63–16.8 μM range values), while neolignans having a C-2′ hydroxyl group (like **4**, **10** and **13**) showed higher AA-antagonism. In addition, if the C-4′ hydroxyl group was oriented toward aryl group (like **2**–**3**, **8**) the PAF-inhibition was slightly increased (IC_{50} in the 1.09–1.37 μM range values).

Kadsurenone **25**, a recognized PAF-antagonist from the Chinese medicinal plant *Piper futokadsura*,[28,29] was also isolated from *N. amazonum*. In addition to the excellent inhibition to PAF, **25** showed activity for AA and ADP at different levels. Interestingly, the activity of mirandin A **20**, which solely differs from **25** by an aromatic *O*-methyl group, was lower to that of **25**. Furthermore, the configuration was found to be an important factor for inhibition of platelet aggregation, since the diastereomer denudatin B **24** exhibited activity significantly lower to that of **20**. The benzofuran-type 8.5′,7.O.4′-connected neolignans **15**–**19**, **21**–**23** and **26** exhibited no significant activity for platelet aggregation. Additionally to kadsurenone **25**, cinerins B–C **2**–**3**, and nectamazin A **8** were

Table 3
Inhibitory effects[a] of neolignans **1**–**26** on the aggregation of rabbit platelets induced by PAF, AA and ADP

Compds	PAF (7.20 nM)[a]	AA (100 μM)[a]	ADP (4.00 μM)[a]
1	16.8 ± 1.6	75.6 ± 1.6	88.9 ± 4.5
2	1.51 ± 0.35	42.8 ± 1.5	>999
3	1.09 ± 0.23	85.8 ± 1.1	>999
4	6.63 ± 0.75	25.6 ± 0.9	356 ± 33
5	3.08 ± 0.15	45.6 ± 2.3	636 ± 25
6	3.03 ± 0.45	38.8 ± 3.2	725 ± 58
7	2.34 ± 0.66	48.6 ± 1.8	>999
8	1.37 ± 0.45	68.7 ± 1.0	>999
9	1.72 ± 0.47	43.4 ± 0.8	>999
10	3.78 ± 0.12	21.6 ± 0.5	>999
11	2.33 ± 0.23	54.6 ± 1.2	>999
12	7.47 ± 0.89	78.9 ± 0.9	521 ± 63
13	6.79 ± 0.78	23.2 ± 2.1	121 ± 38
14	1.61 ± 0.15	2.62 ± 1.03	23.1 ± 1.1
15	460 ± 13	426 ± 15	>999
16	488 ± 22	290 ± 22	54.6 ± 2.3
17	565 ± 25	326 ± 32	56.7 ± 3.4
18	>999	489 ± 28	112 ± 12
19	>999	556 ± 45	165 ± 25
20	3.28 ± 0.31	38.7 ± 1.7	121 ± 18
21	>999	568 ± 35	102 ± 11
22	847 ± 32	425 ± 56	98.3 ± 2.1
23	65.3 ± 9.8	222 ± 33	75.6 ± 3.2
24	75.6 ± 1.3	58.3 ± 2.8	148 ± 16
25	0.153 ± 0.018	5.61 ± 0.98	38.1 ± 2.8
26	18.4 ± 3.3	145 ± 19	124 ± 27
Aspirin	10.3 ± 1.8	5.33 ± 0.45	545 ± 36
Gingkolide B	0.925 ± 0.097	75.6 ± 1.0	>999

[a] The data were expressed as means 95% confidence intervals of four rabbits.

the most potent PAF-antagonists. Compound **14** were the most potent inhibitor for platelet aggregation induced by PAF, AA and ADP.

In summary, a set of 26 naturally-occurring neolignans, related to the bicyclo[3.2.1]octane and 8.5′,7.O.4′-connected classes, were evaluated in order to establish their ability to inhibit COX-1, COX-2, 5-LOX and platelet aggregation induced by PAF, AA and ADP. In the case of bicyclooctanes, the macrophyllin-type neolignans **2**–**3**, **5**–**7**, **11**, **14** as well as the guianin-type neolignan **14** exhibited selectivity toward COX-1 inhibition, whilst compounds **4**, **10** and **13** showed selective COX-2 inhibition, being the activity notably influenced by the configuration of the bicyclootane part. In addition, it was found that the neolignans having a dihydrobenzofuran core (**16**–**19**, **23**, **26**) selectively inhibited the COX-2 isozyme and the levorotatory were found to be slightly more active than dextrorotatory dihydrobenzofurans. Compound **15** was the most active COX-2 inhibitor (whose IS was higher than celecoxib), and compound **10** had a dual 5-LOX/COX-2 inhibition. Macrophyllin-type bicyclooctane neolignans **1**–**13** exhibited a noticeable selectivity toward PAF-inhibition. These results imply that the neolignans isolated from *P. cinereum, O. macrophylla* and *N. amazonum* might be beneficial in the treatment of inflammatory and vascular diseases.

Acknowledgment

We thank the Division de Investigación Sede Bogotá (DIB) (Call 2009, Project Code No. 8003383) at Universidad Nacional de Colombia, for the financial support.

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